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Aerosol from Tobacco Heating System 2.2 has reduced impact on mouse heart gene expression compared with cigarette smoke

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ABSTRACT

Experimental studies clearly demonstrate a causal effect of cigarette smoking on cardiovascular disease. To reduce the individual risk and population harm caused by smoking, alternative products to cigarettes are being developed. We recently reported on an apolipoprotein E-deficient ($Apoe^{-/-}$) mouse inhalation study that compared the effects of exposure to aerosol from a candidate modified risk tobacco product, Tobacco Heating System 2.2 (THS2.2), and smoke from the reference cigarette (3R4F) on pulmonary and vascular biology. Here, we applied a transcriptomics approach to evaluate the impact of the exposure to 3R4F smoke and THS2.2 aerosol on heart tissues from the same cohort of mice. The systems response profiles demonstrated that 3R4F smoke exposure led to time-dependent transcriptomics changes (False Discovery Rate (FDR) < 0.05; 44 differentially expressed genes at 3-months; 491 at 8-months). Analysis of differentially expressed genes in the heart tissue indicated that 3R4F exposure induced the down-regulation of genes involved in cytoskeleton organization and the contractile function of the heart, notably genes that encode beta actin (Actb), actinin alpha 4 (Actn4), and filamin C (Flnc). This was accompanied by the downregulation of genes related to the inflammatory response. None of these effects were observed in the group exposed to THS2.2 aerosol.

1. Introduction

Heart failure affects over 15 million people in Europe (Dickstein et al., 2008) and over 5 million individuals in the United States (Mozaffarian et al., 2015), and is one of the leading causes of hospitalization and morbidity (Sequeira et al., 2014). Heart failure is characterized by, and clinically defined as, the inability of the heart to supply adequate blood perfusion to organs and tissues. Although heart failure is a serious complication of atherosclerosis, other stressors such as diabetes, hypertension, and toxic compounds (Dobrin and Lebeche, 2010) can impact cardiac contraction and favor the development of cardiomyopathy, eventually causing heart failure. It has been reported that these cardiomyopathies show alterations in cardiac muscle structure and function, which could lead to heart failure (Boda et al., 2009; Kamisago et al., 2000). Smoking is a primary cause of the high incidence of cardiovascular diseases (CVD) and is highly associated with endothelial dysfunction (Rahman and Laher, 2007), atherosclerosis (Ambrose and Barua, 2004), and heart failure (Sandhu et al., 2012). Cigarette smoke (CS) contains many known harmful and potentially harmful constituents (Rodgman and Perfetti, 2014), and a large number of them have been shown to promote the development of cardiovascular pathologies (Office of the Surgeon General, 2010).

While the effect of CS on atherosclerosis plaque development and progression is well documented (Phillips et al., 2016; Schleef et al., 2006), the association between smoking, arrhythmia, and cardiomyopathy is less clearly described. Animal models have provided insight into the impact of smoking on heart tissue. Specifically, CS exposure was shown to cause the modulation of cardiac genes involved in cardiac hypertrophy in several CS-exposed animal models (Hu et al., 2013; Al-Arifi et al., 2012; Schleef et al., 2006), and affected the contractile function and cardiac output independent of atherosclerosis. Other studies have shown that CS

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Abbreviations

Apoe$^{-/-}$	Apolipoprotein E Deficient Mouse
THS	Tobacco Heating System
3R4F	Reference Cigarette
CS	Cigarette Smoke
FDR	False Discovery Rate
IPA	Ingenuity Pathway Analysis
GSA	Gene Set Analysis
ORA	Over-Representation Analysis
CVD	Cardiovascular Diseases
COPD	Chronic Obstructive Pulmonary Disease
ECM	Extracellular Matrix
CO	Carbon Monoxide
PAH	Polycyclic Aromatic Hydrocarbons
NF-KB	Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells
NFKBIA	Nuclear Factor of Kappa Light Polypeptide Gene Enhancer In B-cells Inhibitor, Alpha
HPHC	Harmful and Potentially Harmful Constituents
RRP	Reduced-Risk Products ("RRPs") is the term we use to refer to products that present, are likely to present, or have the potential to present less risk of harm to smokers who switch to these products versus continued smoking. We have a range of RRPs in various stages of development, scientific assessment and commercialization. Because our RRPs do not burn tobacco, they produce far lower quantities of harmful and potentially harmful compounds than found in cigarette smoke

exposure increased infarct size in a rat model (Zhu et al., 1994) and favored heart physiological disturbances (Akbarzadeh et al., 2014). Together these data suggest that CS could affect cardiac function and could have an impact on the ultrastructure of cardiomyocytes, leading to cardiomyopathy and thereby enhancing the risk of coronary heart failure.

Although, smoking cessation is one of the pillars of tobacco harm reduction, smoking cessation is difficult for many smokers. Though many smokers are interested in and attempt to quit, the rates of long-term smoking cessation remain very low. According to the United States Surgeon General, (US Department of Health and Human Services, 2010) although about 45% of smokers quit for a day, only approximately 5% succeed in achieving long-term abstinence for one year or more (Office of the Surgeon General, 2010). Consequently, there is a need for alternatives to reduce harm and smoking-related disease for over 40 million American smokers and one billion smokers worldwide. In this context, the availability of potential RRP that yield markedly reduced levels of harmful and potentially harmful constituents (HPHCs) (Schaller et al., 2016) could reduce their harm and risk of tobacco-related disease by drastically reducing smokers' exposure to harmful toxicants in a manner similar to smoking cessation (Phillips et al., 2016). One such product, the Tobacco Heating System 2.2 (THS2.2), which heats the tobacco instead of burning it, was developed. THS2.2 generates an aerosol that mainly contains water, glycerin, nicotine and tobacco flavours (Phillips et al., 2016; Schaller et al., 2016). The electronically controlled heating system avoids tobacco burning which substantially decreases the levels of harmful and potentially harmful constituents (HPHC) emissions, such as carbon monoxide (CO), carbonyls, and polycyclic aromatic hydrocarbons (PAH) (Part of the results are presented in Fig. 1A). To investigate the relative impact of exposure to an aerosol from THS2.2 compared with smoke from the 3R4F reference cigarette (CS), as well as the impact of cessation or switching to THS2.2 after two months exposure to 3R4F (Fig. 1B), we have conducted a whole body inhalation study on Apoe$^{-/-}$ mice. A comparative analysis of chemical composition as well as the physical characteristics for THS2.2 and 3R4F aerosol was performed and had been described elsewhere (Phillips et al., 2016). Briefly, chemical analysis demonstrated that, at the same nicotine concentration (nicotine concentration matched to CS, 29.9 mg/m^3), HPHC and disease end points such as atherosclerosis progression, pulmonary inflammation, and emphysema were substantially reduced in THS2.2-exposed mice compared with 3R4F-exposed mice (Fig. 1A–C) (Elamin et al., 2016; Phillips et al., 2016; Titz et al., 2015), whereas the nicotine and the cotinine levels were comparable in 3R4F and THS2.2 exposure. Because there is substantial evidence for smoking as a risk factor for the development of cardiovascular pathologies, we also analysed heart tissue of Apoe$^{-/-}$ mice from this study. A transcriptomics approach was chosen to identify at least some of the molecular mechanisms underlying the biological effects of exposure to 3R4F and THS2.2 on the heart. We demonstrate that exposure to 3R4F affects specific gene expression in the heart, suggesting disturbances in its cytoskeleton organisation and contractile function. Exposure to THS2.2 aerosol at matching nicotine concentrations did not elicit a significant transcriptional response in the heart, as seen in the lower impact on differential gene expression (491 differentially expressed genes at eight months of 3R4F CS exposure, no significantly differentially expressed genes for THS2.2 at eight months of THS2.2 exposure).

2. Materials and method

2.1. Study design

All procedures involving animals were performed in an Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)-accredited, Agri-Food & Veterinary Authority of Singapore-licensed facility in compliance with the National Advisory Committee for Laboratory Animal Research Guidelines on the Care and Use of Animals for Scientific Purposes, as described previously (Phillips et al., 2016).

Female Apoe$^{-/-}$ mice, (age 8–10 weeks at the beginning of the protocol) were exposed to 3R4F CS (University of Kentucky; https://ctrp.uky.edu/) or to THS2.2 aerosol for up to 8 months (Fig. 1B). Briefly, mainstream CS from 3R4F cigarettes was generated on 30-port rotary smoking machines, while aerosol from THS2.2 sticks was generated on modified 30-port rotary smoking machines equipped with the respective stick holders. 3R4F cigarettes were smoked and aerosol from THS2.2 sticks was generated according to the Health Canada Intensive Smoking Protocol based on ISO standard 3308 (revised in 2000), as described previously (Phillips et al., 2016).

Diluted mainstream smoke extracted from 3R4F cigarettes (600 mg total particulate material/m^3, equivalent to 29.9 mg nicotine/m^3), THS2.2 aerosol (nicotine-matched to 3R4F, 29.9 mg/m^3), or filtered air were used to expose mice (whole body exposure), during 3 h per day, 5 days per week, for up to 8 months. To avoid a buildup of excessive carboxyhemoglobin concentrations in the 3R4F group, intermittent daily exposure to fresh filtered air for 30 min after the first hour of smoke exposure and for 60 min after the second hour of exposure was provided. Apoe$^{-/-}$ mice exposed to fresh air (sham) were used as a control group. After 2 months of 3R4F exposure, subsets of mice were exposed to fresh air to mimic smoking cessation or were switched to THS2.2.